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A New Species, *Pueraria xyzhuii* (Leguminosae) from Yunnan, China, with Pollen Stainability and Pollen Morphology in Comparison to Related Species

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A new species of *Pueraria*, *P. xyzhuii* H. Ohashi & Iokawa, is described from Yunnan on the basis of a specimen, Henry 13626 (A). Its pollen stainability was examined, discounting its presumable hybrid origin. The species is most similar to *P. montana*, but differs from it in having small leaves, 3-lobed leaflets, large branched inflorescences, long auriculate keel-petals, and perforate sculpture in pollen grains. The perforate sculpture is recognized for the first time in the genus. Pollen grains of *P. xyzhuii* are described and compared with its related species, *P. bouffordii*, *P. edulis* and *P. montana* (var. *lobata*, var. *montana* and var. *chinensis*).

Key words: China, Leguminosae, new species, pollen grains, *Pueraria*.

In his revision of the genus *Pueraria*, Maesen (1985) noted that two specimens, Henry 13626 kept in BM, NY and US and Henry 10931 in CAL, K and NY, are referable to *Pueraria* but not to any known species of the genus. He recognized them to as being near to *P. lobata* and *P. edulis* and considered that they are *P. montana* var. *lobata* (as *P. lobata* var. *lobata*) or a supposed hybrid between *P. montana* var. *lobata* (as *P. lobata* var. *lobata*) and *P. edulis* Pamp., or *P. montana* var. *lobata* (as *P. lobata* var. *montana*). Relations between these names are confusable, but the correct names for *P. lobata* var. *lobata* and var. *montana* are *P. montana* var. *lobata* and var. *montana*, respectively (Maesen 1994), although we consider that *P. lobata* is distinct from *P. montana* and that *P. montana* var. *chinensis* is recognized as *P. lobata* subsp. *thomsonii* (Benth.) H. Ohashi & Tateishi (Ohashi et al. 1988). In this paper, we adopt the names of *P. montana* in Maesen's concept (1994) for convenience. Maesen (1985) regarded Henry 13626 and Henry 10931 as identical, but we recognized the former differs from the latter in having branched inflorescences and sharply lobed leaflets. Henry 10931 is unique in the shape of leaflets in the genus. These two specimens are considered to be different taxa in this paper.

We had the chance to examine a specimen Henry 13626 kept in A (Figs. 1, 2) that is recognized as a duplicate of the Henry 13626 (BM, NY and US) examined by Maesen (1985). The specimen Henry 13626 (A) is, however, different from *P. montana* including var. *lobata* and var. *montana* as was already pointed out by Ohashi (2005) under a note for *P. bouffordii*. Also, Henry 13626 differs from *P. edulis* as mentioned by Maesen (1985). Moreover, the specimen differs from *P. montana* and *P. edulis* in the calyx with the lowest lobe slightly longer than the lateral lobes (distinctly longer in *P. montana* and *P. edulis*) and with acute apex (acuminate in *P. montana* and *P. edulis*), and the flower with almost equally sized wings and keel-petals (keel-petals larger than the wings in *P. montana* and *P. edulis*). Henry 13626 (A) is morphologically distinct from *P. montana* and *P. edulis*.

The specimen is also somewhat similar to *Pueraria bouffordii* in having densely flowered pseudoracemes with 2–3 flowers per node and a calyx with all the lobes almost equal in length (Ohashi 2005).

We observed pollen grains taken from Henry 13626 (A) to compare morphology with related species and to examine the possibility of a hybrid origin of the specimen. The pollen grains of the specimen have perforate sculpture that found in the genus for the first time, although the sculpture pattern is clearly related to reticulate pattern of the genus. The pollen viability was tested by anilineblue lactophenol method (Hauser and Morrison 1964). Ratio of stained grains is 68.3 % (total sample grains: 1511, positively stained grains: 1032) in the specimen. The result shows about 30 % abortive pollen grains in the specimen (Fig. 3a). We also examined pollen grains in specimens of *P. edulis* from Yunnan: Iwatsuki et al. 413 (TUS) (Fig. 3b) and *P. montana* var. *lobata* from Yunnan: Henry 11041 (A), Tsai 61170 (A) (Fig. 3c); Hunan: Li 60 (TUS) and Japan, Sendai: Ohba 708251 (TUSG) (Fig. 3d) for a comparison of the ratio between these specimens and Henry 13626. Results of the ratio of stained grains in the specimens examined are shown in Table 1. The ratio of pollen stainability shows variation in these specimens. We, therefore, did not consider the specimen, Henry 13626 (A), to be a hybrid between *P. lobata* and *P. edulis*.

Morphologically, the specimen Henry 13626 (A) differs from all the known species of the genus in having small leaves, 3-lobed leaflets, large branched inflorescences, keel-petals with long auricles at the base of the lamina, and perforate pollen grains. We therefore consider the specimen is representing a new species, and name it *P. xyzhuii* in honor of Dr. Xiangyun Zhu, a Chinese legume taxonomist in PE.

Pueraria xyzhuii H. Ohashi & Iokawa, sp. nov. [Figs. 1, 2]

A *P. lobata* foliis parvis, foliolis 3-lobis, inflorescentiis magnis remosis, carina longiauriculatis, granulis pollinis perforatis differt.

Typus. China. Yunnan. Mengtze, 5000ft. A. Henry 13626 (A).

Vine. Stem herbaceous, densely reflexed appressed sericeous. Stipules peltate, 3–4 mm wide. Leaves pinnately trifoliolate, 16–19 cm long, upper surface appressed short sericeous on nerves, underside uniformly sericeous, lateral nerves prominent on under surface, 5–7 each side, extending to margin; terminal leaflet broadly ovate, to 8 cm long, 6.5 cm wide, 3-lobed, entire along margin, apex acute, base broad-truncate to rounded, with a pulvinus 3 mm long; rachis 2–3 cm long, with a pair of stipels at the top; lateral leaflets obliquely broadly ovate, 3-lobed, almost equal to the terminal one in size, 7–8 cm long, 5–7 cm wide, apex acute, base rounded, with a pulvinus 4–6 mm long, with one stipel at the base of each pulvinus; petiole striate, 7–8 cm long, with appressed ascending sericeous hairs; stipels narrowly ovate-triangular, 4–5 mm long. Inflorescence pseudoraceme, branched, probably to 46 or 48 cm long, bractate at base, 2(–3)-flowered at a node, internodes irregular, 1–20 mm long; rachis nodose with small nodules, densely appressed sericeous. Bracts deciduous. Pedicels 3–4 mm long, appressed and patent sericeous. Bracteoles 2 at the top of pedicel, narrowly ovate, 3 mm long, 1 mm wide, densely sericeous outside and margin, glabrous inside. Calyx campanulate, 5–5.5

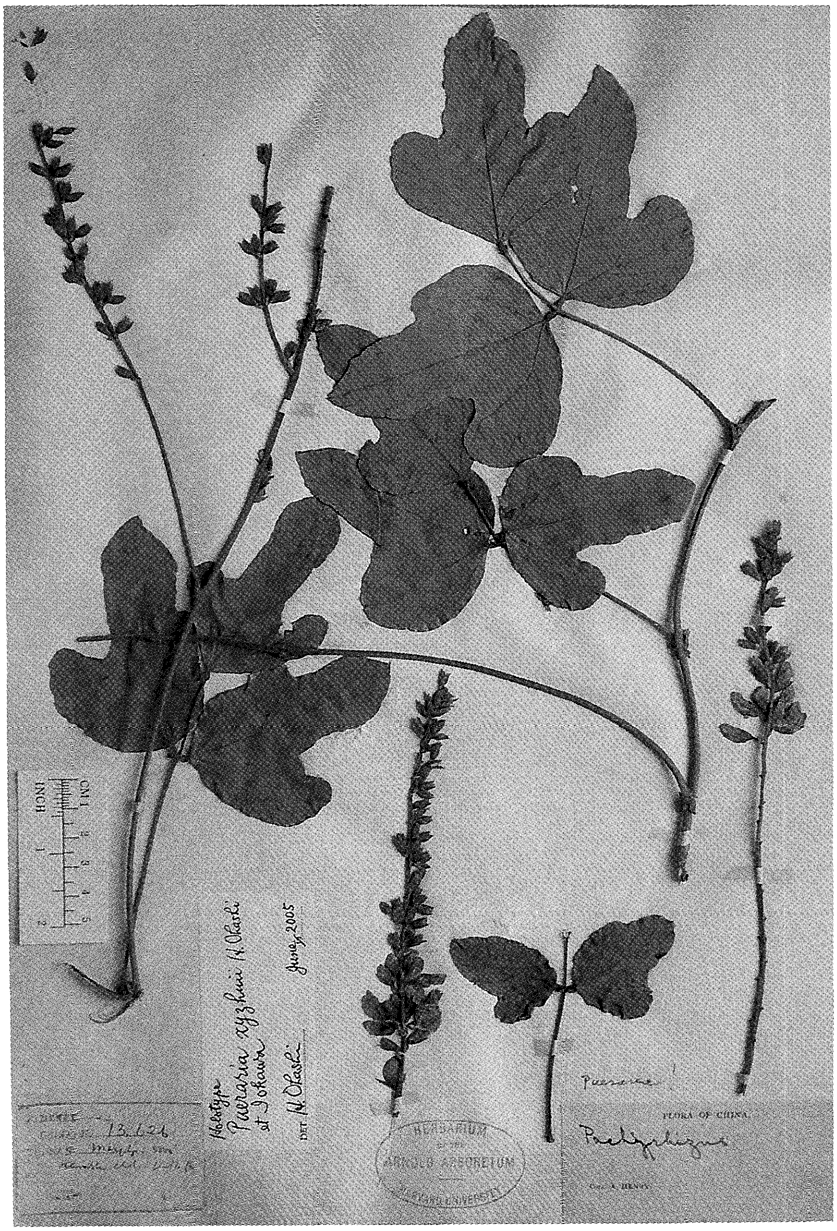

Fig. 1. Henry 13626 (A), holotype of *Pueraria xyzhuii* H. Ohashi & Iokawa.

mm long, densely appressed sericeous outside, appressed sericeous inside, 5 lobed, tube ca. 2 mm long; upper 2 lobes almost entirely connate to the top, ca. 4.5 mm long, 4 mm wide at base, obtuse at apex; lateral lobes narrowly ovate, 4 mm long, 2.2 mm wide, apex acute; lowest lobes narrowly ovate, acute at apex, 4.5 mm long, 2 mm wide at base. Standard glabrous, clawed at base, banner circular to transversely broadly

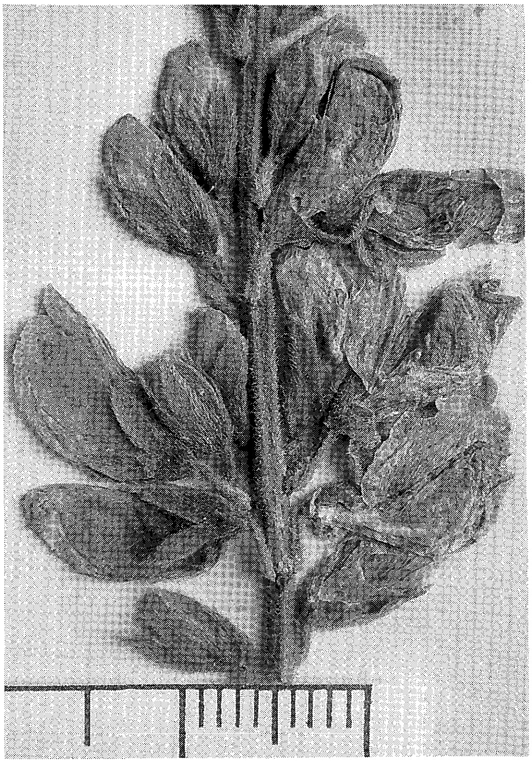

Fig. 2. Part of inflorescence of Henry 13626 (A).

elliptic, ca 11 mm long, 13 mm wide, apex more or less emarginate, base slightly cordate, callous, claw ca. 2 mm long, 1 mm wide; wings shallowly incurved, glabrous, ca. 12.5 mm long, banner elliptic to semicircular, 10 mm long, 4 mm wide, obtuse at apex, with a small auricle 1 mm long at base, connate to the keel-petals at slightly basal portion of central banner, claw ca. 3 mm long; keel-petals incurved, glabrous, almost equal to the wing, ca. 12 mm long, banner elliptic, 9.5 mm long, 4 mm wide, apex obtuse, auriculate (auricles ca. 2 mm long), joined along abaxial margin, claw ca. 3 mm long. Stamens monadelphous, ca. 10.5 mm long, upper part incurved at right angle, with minute openings at both sides of the vexillary stamen at base. Pistil sessile, elongate, flattened, ca. 10.5 mm long, without disk at base; ovary appressed sericeous, style continuous from the ovary, incurved at about right angle at about the middle; stigma terminal, minutely globose, penicillate around base. Pods unknown.

Pollen morphology of *Pueraria xyzhuii* and its related species

Pollen morphology of *Pueraria xyzhuii* was observed in comparison to its related species, *P. bouffordii*, *P. edulis* and *P. montana* including var. *montana*, var. *lobata* and var. *chinensis*.

Materials and methods: Pollen grains were taken from herbarium specimens kept in TUS, except *Pueraria xyzhuii, P. bouffordii* and *P. montana* var. *lobata* from A. Pollen grains were acetolysed following the standard method and dehydrated in an ethanol series (Erdtman 1960, Ye and Ohashi 2002). For SEM observation, pollen grains were air dried and coated with gold palladium, and examined with a scanning electron microscope (JSM-5600LV, JEOL) in Joetsu

Table 1. Pollen stainability of *Pueraria xyzhuii, P. edulis* and *P. montana* var. *lobata*

Taxon	Ratio of stained grains	Number of total grains observed	Number of stained normal grains
P. xyzhuii (Fig. 3a)	68.70 %	1511	1032
P. edulis (Fig. 3b)	67.70 %	1891	1281
P. montana var. *lobata*: Tsai 61170	96.80 %	1713	1659
P. montana var. *lobata*: Li 60	97.60 %	1526	1489
P. montana var. *lobata*: Ohba 708251	50.30 %	1753	881

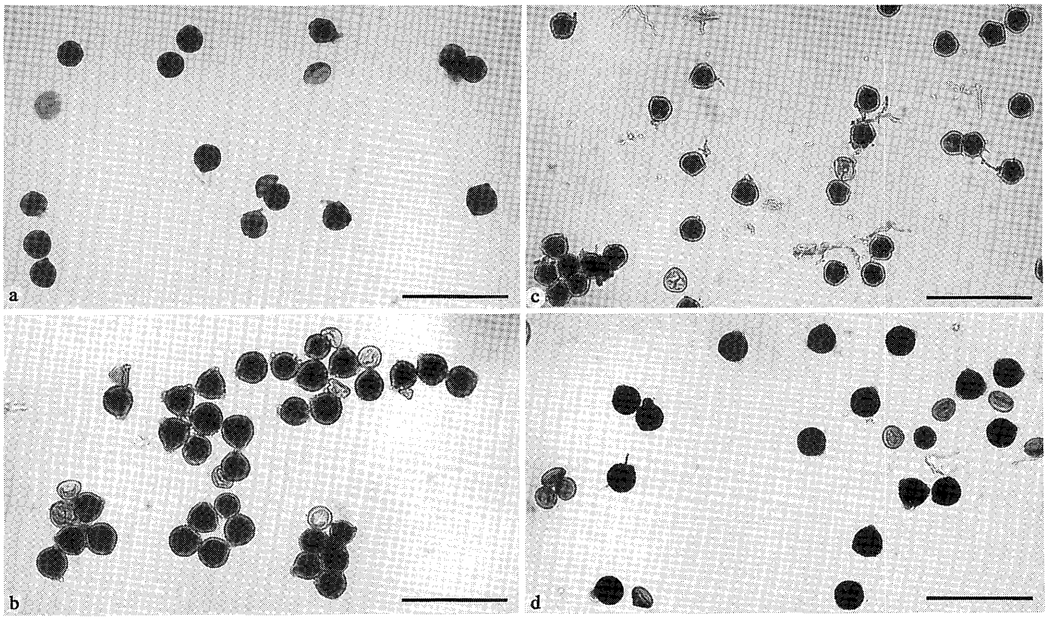

Fig. 3. Stained pollen grains of *Pueraria xyzhuii* (a), *P. edulis* (b) and *P. montana* var. *lobata* from Yunnan, Tsai 61170 (c) and Japan, Ohba 708251(d). Scale bar = 0.1 mm.

University of Education. The polar and equatorial lengths were measured on 30 grains in each sample. Pollen terminology generally follows Punt et al. (1994) and Ohashi et al. (2005).

Pueraria xyzhuii H. Ohashi & Iokawa (Figs. 4a–c).

Pollen grains tricolporate; (23.3–)25.8 (–28.3) μm in polar axis, (20.9–)24.0(–27.8) μm in equatorial diameter; P/E = (1.00–)1.08 (–1.21), prolate spheroidal to subprolate in shape, semiangular in polar view. Colpi 0.65–0.75 times the length of polar axis, narrowing slightly to pointed ends, colpus membrane smooth and partly granulate, margins undifferentiated from the mesocolpium or ± smooth and a little pitted. Sexine perforate in mesocolpium, smooth in apocolpium.

Specimen examined: China, Yunnan. Henry 13626 (A).

Pueraria bouffordii H. Ohashi (Figs. 5d–f).

Pollen grains tricolporate; (22.9–)24.5 (–26.1) μm in polar axis, (21.6–)23.1(–25.2) μm in equatorial diameter; P/E = (1.00–)1.06 (–1.15), prolate spheroidal to subprolate in shape, semiangular in polar view. Colpi ca. 0.8 times the length of polar axis, narrowing slightly to pointed ends, colpus membrane smooth and partly granulate, margins undifferentiated from the mesocolpium or ± smooth and a little pitted. Sexine reticulate in mesocolpium, perforate in apocolpium; muri 0.2–1.1 μm wide; lumina 0.2–1.3 μm in diameter, with granules.

Specimen examined: China, Guizhou. Sino-American Guizhou Botanical Expedition 1031 (A).

Pueraria edulis Pamp. (Figs. 4g–i).

Pollen grains tricolporate; (21.6–)23.9 (–26.4) μm in polar axis, (16.6–)20.3(–23.9) μm in equatorial diameter; P/E = (1.05–)1.18

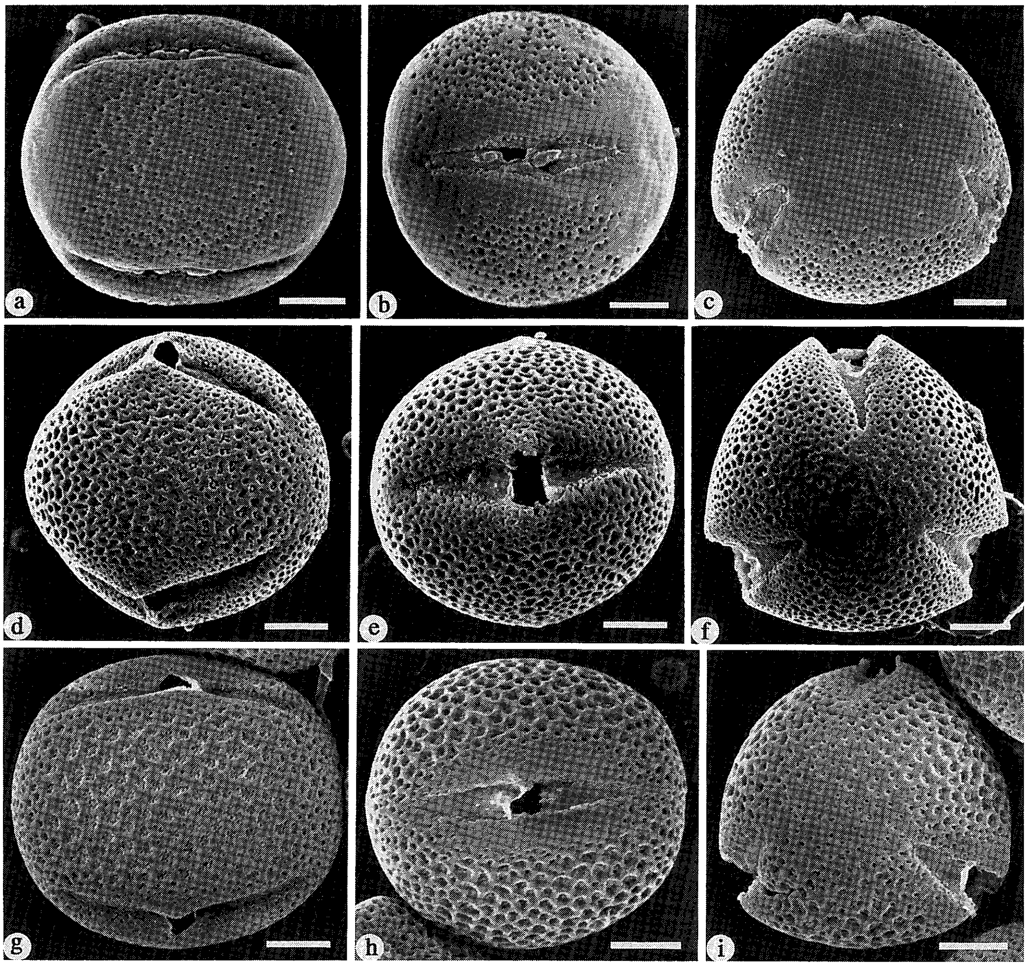

Fig. 4. Pollen grains of *Pueraria xyzhuii* (a–c), *P. bouffordii* (d–f) and *P. edulis* (g–i). a, d, g: Equatorial view showing mesocolpium. b, e, h: Equatorial view showing aperture. c, f, i: Polar view. Scale bar = 5 µm.

(–1.45), prolate spheroidal to prolate in shape, semiangular in polar view. Colpi 0.65–0.75 times the length of polar axis, narrowing slightly to pointed ends, colpus membrane smooth and partly granulate, margins undifferentiated from the mesocolpium or ± smooth and a little pitted. Sexine reticulate in mesocolpium, perforate in apocolpium; muri 0.4–1.0 µm wide; lumina 0.1–1.2 µm in diameter, with granules.

Specimen examined: China, Yunnan. Iwatsuki & al. 413. (TUS).

Pueraria montana (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida (Figs. 5a–c).

Pollen grains tricolporate; (22.1–)23.7 (–24.6) µm in polar axis, (20.3–)21.6(–22.8) µm in equatorial diameter; P/E = (1.02–)1.10 (–1.20), prolate spheroidal to subprolate in shape, semiangular in polar view. Colpi 0.7–0.8 times the length of polar axis, narrowing slightly to pointed ends, colpus membrane smooth and partly granulate, margins undifferentiated from the mesocolpium or ±

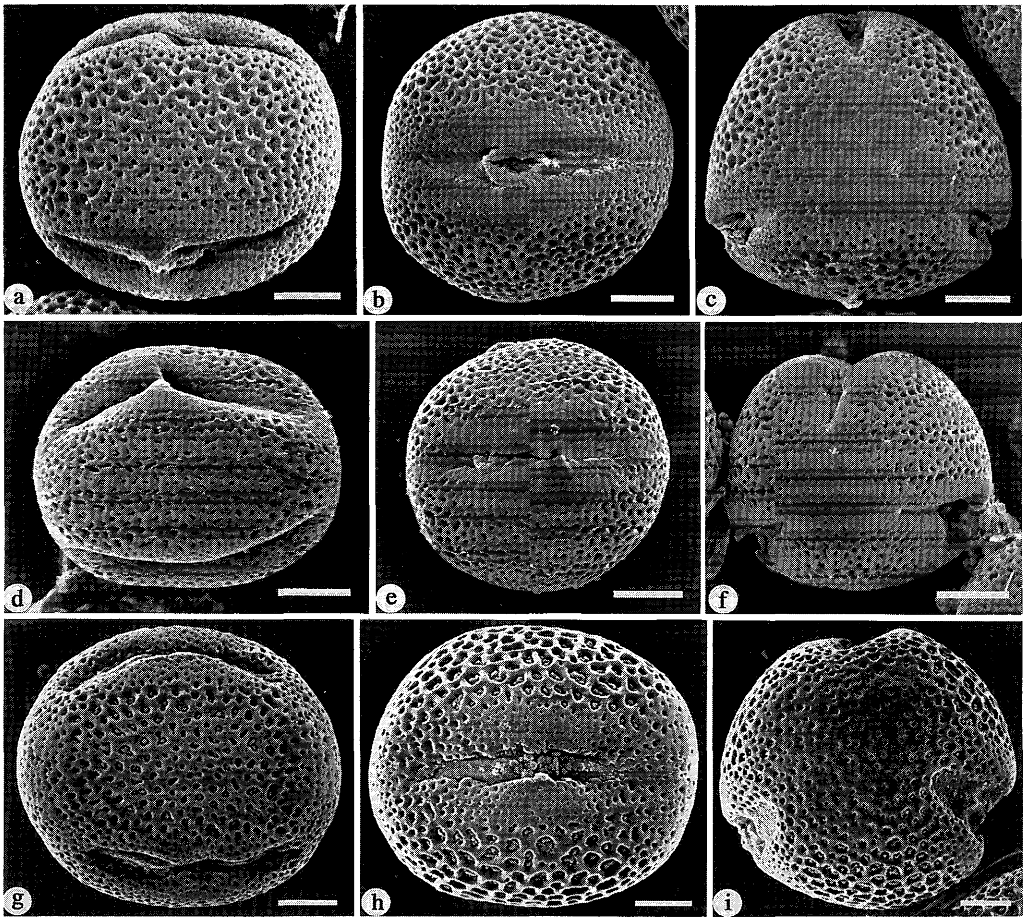

Fig. 5. Pollen grains of *Pueraria montana* var. *lobata* (a–c), var. *montana* (d–f) and var. *chinensis* (g–i). a, d, g: Equatorial view showing mesocolpium. b, e, h: Equatorial view showing aperture. c, f, i: Polar view. Scale bar = 5 μm.

Table 2. Pollen grains of *Pueraria xyzhuii* and related species

Taxon	Polar length	Equatrial diameter	Sculupture	Muri	Lumina
P. xyzhii	(23.3–)25.8(–28.3)	(20.9–)24.0(–27.8)	perforate		
P. boufffordii	(22.9–)24.5(–26.1)	(21.6–)23.1(–25.2)	reticulate	0.2–1.1	0.2–1.3
P. edulis	(21.6–)23.9(–26.4)	(16.6–)20.3(–23.9)	reticulate	0.4–1.0	0.1–1.2
P. montana var. *lobata*	(22.1–)23.7(–24.6)	(20.3–)21.6(–22.8)	reticulate	0.5–1.2	0.2–1.5
P. montana var. *montana*	(18.7–)19.9(–21.4)	(15.3–)17.4(–20.0)	reticulate	0.2–0.7	0.2–1.1
P. montana var. *chinensis*	(27.0–)29.4(–34.9)	(29.9–)25.6(–30.3)	reticulate	0.5–1.3	0.2–2.3

smooth and a little pitted. Sexine reticulate in mesocolpium, perforate in apocolpium; muri 0.5–1.2 µm wide; lumina 0.2–1.5 µm in diameter, with granules.

Specimen examined: China, Yunnan. A. Henry 11041 (A); Yunnan. Ping-pien Hsien, alt. 1200 m. 24 July 1934. H. T. Tsai 61170 (A). Hunan. Qidongxian, Lingguanzhen. 24 Aug. 2002. J. D. Li 60 (TUS). Japan, Sendai. 26 Aug. 1970. H. Ohba 708251 (TUSG).

Pueraria montana (Lour.) Merr. var. *montana* (Figs. 5d–f).

Pollen grains tricolporate; (18.7–)19.9 (–21.4) µm in polar axis, (15.3–)17.4(–20.0) µm in equatorial diameter; P/E = (1.06–)1.15 (–1.34), prolate spheroidal to subprolate in shape, semiangular in polar view. Colpi 0.7–0.8 times the length of polar axis, narrowing slightly to pointed ends, colpus membrane ± smooth and partly verrucate, margins undifferentiated from the mesocolpium or ± smooth and a little pitted. Sexine reticulate in mesocolpium, perforate in apocolpium; muri 0.2–0.7 µm wide; lumina 0.2–1.1 µm in diameter, with granules.

Specimen examined: Taiwan, Pingtung Co., Mt. Kaoshihfo-shan. 27 Oct. 1982. Ohashi & al. 13566 (TUS).

Pueraria montana var. *chinensis* (Ohwi) Maesen & Almeida (Figs. 5g–i).

Pollen grains tricolporate; (27.0–)29.4 (–34.9) µm in polar axis, (21.9–)25.6(–30.3) µm in equatorial diameter; P/E = (1.04–)1.15 (–1.33), prolate spheroidal to subprolate in shape, semiangular in polar view. Colpi 0.6–0.7 times the length of polar axis, narrowing slightly to pointed ends, colpus membrane smooth and partly granulate, margins undifferentiated from the mesocolpium or ± smooth and a little pitted. Sexine reticulate in mesocolpium, perforate in apocolpium; muri 0.5–1.3 µm wide; lumina 0.2–2.3 µm in diameter, with granules.

Specimen examined: Taiwan. Tainan Co., Henglu. 15 Oct. 1988. Ohashi & al. 24070 (TUS).

Results of the present examination of pollen grains are summarized in Table 2. Pollen grains of *Pueraria xyzhuii* are distinct in sculpture from the related species, but are close to them in other pollen characters. *Pueraria montana*, however, shows clear diversity in pollen size among the three varieties (Taki et al. 1977, Ohashi et al. 2005). Pollen of var. *montana* is distinctly smaller than that of var. *chinensis*, and that of var. *lobata* is intermediate between them.

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大橋広好[a]，五百川　裕[b]：中国雲南省産マメ科クズ属の一新種と近縁種の花粉形態

クズ属のモノグラフを発表した Maesen (1985) は中国雲南省で採集された Henry 13626 (BM, NY, US) という標本をクズ *Pueraria lobata*，またはクズと *P. edulis* Pamp. の雑種，またはタイワンクズのいずれかであろうと同定した．Ohashi (2005) は中国雲南省から *Pueraria bouffordii* H. Ohashi を記載した際にハーバード大学アーノルド樹木園所蔵の重複標本の一枚（Henry 13626, A）を検討して新種ではないかとし，さらに検討の必要があることを指摘した．本研究ではまず，これまでに知られたクズ属の種と Henry 13626 (A) の形態を比較した．Henry 13626 (A) は葉が小さく，花序は多分大型で，その基部から 2–3 本に枝分かれすることで既知種とは異なっていた．次に，Henry 13626 の花粉染色率を調べた結果，正常花粉は約 70 % であった．クズと *P. edulis* の花粉染色率と比較し，この標本の植物は雑種とは考えられないと判断した．さらに Henry 13626 の花粉粒は微穿孔型 perforate であることが明らかになった．これまでに知られたクズ属の花粉は全て網目型 reticulate であり，この花粉表面模様は属として新しい型である．以上の結果に基づいて Henry 13626 (A) の植物を新種と認め，*Pueraria xyzhuii* H. Ohashi & Iokawa と命名した．種形容語は東北大学でマメ科を研究し学位を得た中国科学院植物研究所の朱　相雲 Zhu Xiang-yun 博士に献名した．

さらに，本論文では *Pueraria xyzhuii* とその近縁種である *P. bouffordii*, *P. edulis* およびクズ（タイワンクズとシナクズを含む）の花粉形態を比較した．しかし，花粉表面模様以外には非常によく似た花粉形態であった．クズの花粉については生薬学で詳しく調べられており（瀧他 1977），花粉サイズに違いのあることが報告されている．花粉サイズはタイワンクズ，クズ，シナクズの順に大きくなる．

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